- (a) (i) transfecting the cell with a first vector that expresses a replication factor; or
- (ii) otherwise obtaining a cell that expresses or will express the replication factor; and
 - (b) transfecting the cell with a second vector, wherein
- (i) the second vector contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the [DNA] selectable marker;
- (ii) the second vector additionally contains a second DNA in operative combination with a promoter for expression of the second DNA, and which second DNA does not code for a selectable marker; and
- (iii) extrachromosomal replication of the second vector is dependent upon presence within the cell of the replication factor.
- 3. A method according to Claim 2 wherein the viral replication factor is selected from polyoma large T antigen, EBNA-1 antigen, papilloma virus replication factors, SV40 large T antigen and functional variants, analogues and derivatives thereof appropriate to the cell species.
- 4. A method according to Claim 1 wherein the second vector does not express the replication factor.
- 5. A method according to Claim 1 wherein the selectable marker is an antibiotic resistance gene.
- 6. A method according to Claim 1 further comprising transfecting the cell with a third vector, wherein the third vector contains a DNA, on is adapted to receive a DNA.

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in operative combination with a promoter for expression of the DNA, and replication of the third vector is dependent upon presence within the cell of the replication factor.

- 8. A method according to Claim 1 wherein the cell is selected from the group consisting of a mammalian cell and an axian cell.
 - 9. A method according to Claim 1 wherein the cell is an embryonic cell.
- 11. A method according to Claim 1 for transfection of an ES cell wherein the ES cell of step (a) expresses polyoma large T antigen and the second vector comprises a natural target for polyoma large/T antigen.
- 12. A method according to Claim 1 wherein the DNA codes for a polypeptide or protein.
- 13. A method according to Claim 1 wherein the DNA codes for an antisense RNA.
 - 14. A method according to Claim 1 wherein the promoter is inducible.
- 15. A method according to Claim wherein transcription of the DNA can be activated by a site specific recombinase.
- 16. A method according to Claim 1 wherein replication of the second vector can be prevented by a site specific recombinase.
 - 17. A vector for transfection of a pluripotent cell in vitro, wherein:
- (i) the vector contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker;
- (ii) the vector contains a second DNA in operative combination with a promoter for expression of the DNA, and which second DNA does not code for a selectable marker;

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- (iii) extrachromosomal replication of the vector is dependent upon presence within the cell of a replication factor; and
 - (iv) the vector does not express the replication factor.
- 19. A vector according to Claim 18 wherein the viral replication factor is selected from the group consisting of polyoma large T antigen, EBNA-1 antigen, papilloma virus replication factors, SV40 large T antigen and functional variants, analogues and derivatives thereof.
- 20. A vector according to Claim 17 wherein the vector is [substantially] free of DNA coding for the replication factor or any part thereof.
- 21. A vector according to Claim 17 for transfection of mammalian or avian cells.
 - 22. A vector according to diairn 17 for transfection of ES cells.
- 23. A vector according to Claim 22 comprising a natural target for polyoma large T antigen.
- 24. A vector according to Claim 17 wherein the DNA codes for a polypeptide or protein.
- 25. A vector according to Claim 17 wherein the DNA codes for an antisense DNA.
 - 26. A vector according to Claim 17 wherein the promoter is inducible.
- 27. A vector according to any Claim 17 wherein the selectable marker is an antibiotic resistance gene.
- 29. An ES, EC or EG cell transfected with a first vector that expresses a replication factor and with a second vector according to Claim 17.

- 32. A cell selected from an ES, EC or EG cell according to Claim 29, and differentiated progerly thereof.
- 33. An assay for the effect of presence in a pluripotent cell of a protein or polypeptide or other product of DNA expression, comprising the steps:
- (a) (i) transfecting the cell with a first vector that expresses a replication factor; or
- (ii) otherwise obtaining a cell that expresses or will express the replication factor;
 - (b) transfecting the cell with a second vector, wherein
- (i) the second vector contains a DNA coding for the protein or polypeptide or other product of DNA expression in operative combination with a promoter for expression of the DNA;
- (ii) the second vector also contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker; and
- (iii) extrachromosomal replication of the second vector is dependent upon presence within the cell of the replication factor;
 - (c) selecting for cells that have been transfected with the second vector; and
- (d) maintaining the selected cells over a plurality of generations so as to assay the effect of expression of the protein or polypeptide or other product of DNA expression.
- 35. An assay according to Claim 33 for assay of the effect of presence in the cell of two factors, each factor being independently selected from a protein, a polypeptide and another product of DNA expression.

- 36. A method of screening a library of cDNAs comprising assaying the effect of expression of each of the cDNAs according to the method of Claim 33.
- 37. A method of investigating the properties of a DNA sequence comprising expressing in a pluripotent cell a composite DNA including (a) the DNA sequence under investigation, linked to (b) a DNA coding for a cell active protein, wherein
- (i) activity of the cell active protein is dependent upon transport of the cell active protein to the cell surface,
- (ii) the DNA of (b) does not code for a polypeptide that direct[ing]s transportation of the cell active protein to the cell surface, and
- (iii) the cell active protein inhibits differentiation of the cell and in the absence of the cell active protein the cell will differentiate.
- 39. A method according to Claim 37 wherein the DNA of (b) is obtained by deleting or disabling, from a DNA encoding a cell surface or secreted protein, that portion of the DNA that codes for the polypeptide sequence responsible for transportation of the protein to the cell surface.
- 40. A method according to Claim 37 wherein the cell active protein induces a morphological or proliferative change in the cell.
- 42. A method according to Claim 37 wherein the cell active protein is a cell surface receptor.
- 44. A method according to Claim 37 comprising investigating the properties of a DNA in mammalian or avian cells.
- 45. A method according to Claim 37 comprising investigating the properties of a DNA in embryonic cells.

- 47. A method according to Claim 37 comprising expressing the composite DNA by :
- (a) (i) transfecting the cell with a first vector that expresses a replication factor; or
- (ii) otherwise obtaining a cell that expresses or will express the replication factor;
 - (b) transfecting the cell with a second vector, wherein
- (i) the second vector contains the composite DNA in operative combination with a promoter for expression of the composite DNA;
- (ii) the second vector also contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker; and
- (iii) extrachromosomal replication of the second vector is dependant upon presence within the cell of the replication factor;
 - (c) selecting for cells that have been transfected with the second vector; and
- (d) maintaining the selected cells over a plurality of generations so as to assay the effect of expression of the composite DNA.
- 48. A method according to Claim 37 wherein step (a) is carried out once and the cells obtained are divided and used for a plurality of separate methods in which steps (b)-(d) are carried out a plurality of times with second vectors containing different DNA sequences.
- 49. A method according to Claim 37 for identification of a DNA coding for a cell surface or secreted protein.

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